PATENT COOPERATION TR[illegible]TY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

To:
E.I. DU PONT DE NEMOURS AND COMPANY
Legal/Patent Records Center
Attn. CHRISTENBURY, L.
1007 Market Street
Wilmington, Delaware 19898
UNITED STATES OF AMERICA

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NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION
(PCT Rule 44.1)

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Lmc 11/6/98
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Applicant's or agent's file reference BB1037F	Date of mailing (day/month/year) 27/10/1998
	FOR FURTHER ACTION See paragraphs 1 and 4 below
International application No. PCT/US 98/ 06051	International filing date (day/month/year) 27/03/1998
Applicant E.I. DU PONT DE NEMOURS AND COMPANY et al.	

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicants's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Chantal Meyer TRB NOTED 11.4.98

Form PCT/ISA/220 (January 1994)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
BB1037F		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 98/06051	27/03/1998	27/03/1997
Applicant		
E.I. DU PONT DE NEMOURS AND COMPANY et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [X] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [X] filed with the international application.
   - [ ] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] Transcribed by this Authority

4. With regard to the **title,**
   - [X] the text is approved as submitted by the applicant
   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   - [X] the text is approved as submitted by the applicant
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:
   Figure No. _____
   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.
   - [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/06051

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N9/06 C12N9/12 C12N9/88 C12P13/08 C12N15/82

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C12P

Documentation searched other than minimumdocumentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 19190 A (E.I. DU PONT DE NEMOURS AND COMPANY) 30 September 1993 see page 36, line 22 - page 38, line 20 see page 103, line 25 - page 107, line 18	1,6,8-20
X	WO 95 15392 A (E.I. DU PONT DE NEMOURS AND COMPANY) 8 June 1995 see page 28, line 25 - page 30, line 35 see page 67, line 4 - page 70, line 35	1,6,8-20
X	WO 96 19588 A (MIAMI UNIVERSITY) 27 June 1996 see page 33 - page 45; figure 2	1
P,X	EP 0 771 879 A (AJINOMOTO KK) 7 May 1997 see the whole document	1
X	& WO 95 34672 A	1

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publicationdate of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of theinternational search	Date of mailing of the international search report
13 October 1998	27/10/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	De Kok, A

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	XUAN J-W ET AL: "Overlapping reading frames at the LYS locus un the yeast YARROWIA-LIPOLYTICA" MOLECULAR AND CELLULAR BIOLOGY., vol. 10, no. 9, 1990, pages 4795-4806, XP002021824 WASHINGTON US cited in the application see the whole document	1
A	GONCALVES-BUTRUILLE M ET AL: "Purification and characterization of the bifunctional enzyme lysine-ketoglutarate reductase-saccharopine dehydrogenase from maize" PLANT PHYSIOLOGY., vol. 110 , no. 3, 1996, pages 765-771, XP002079107 ROCKVILLE US cited in the application see the whole document	1
A	WO 89 11789 A (MOLECULAR GENETICS RESEARCH AND DEVELOPMENT LTD PARTNERSHIP) 14 December 1989 see the whole document	1,16-20
A	NEWMAN T ET AL: "GENES GALORE: A SUMMARY OF METHODS FOR ACCESSING RESULTS FROM LARGE-SCALE PARTIAL SEQUENCING OF ANONYMOUS ARABIDOPSIS CDNA CLONES" PLANT PHYSIOLOGY., vol. 106, 1 January 1994, pages 1241-1255, XP000571449 ROCKVILLE US see the whole document	1-5
P,X	CORD-NETO G ET AL: "Lysine-ketoglutarate reductase/saccharopine dehydrogenase bifunctional enzyme from maize seeds" EMBL SEQUENCE DATABASE,21 June 1997, XP002079108 HEIDELBERG DE cited in the application Accession Nr.: AF003551 see abstract	1,2

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International Application No
PCT/US 98/06051

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	EPELBAUM S ET AL: "Lysine-ketoglutarate reductase and saccharopine dehydrogenase from Arabidopsis thaliana: nucleotide sequence and characterization" PLANT MOLECULAR BIOLOGY., vol. 35, no. 6, 1997, pages 735-748, XP002079109 DORDRECHT NL cited in the application see the whole document ---	1-5
P,X	TANG G ET AL: "Regulation of lysine catabolism through lysine-ketoglutarate reductase and saccharopine dehydrogenase in arabidopsis" PLANT CELL., vol. 9, no. 8, 1997, pages 1305-1316, XP002079110 MD US cited in the application see the whole document -----	1-5

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 98/06051

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 9319190 A	30-09-1993	AU	675933 B	27-02-1997
		AU	3923393 A	21-10-1993
		CA	2132414 A	30-09-1993
		EP	0640141 A	01-03-1995
		JP	7504821 T	01-06-1995
		MX	9301521 A	01-09-1993
		US	5773691 A	30-06-1998
		ZA	9301978 A	19-09-1994
WO 9515392 A	08-06-1995	AU	1255995 A	19-06-1995
		BR	9408228 A	26-08-1997
		CA	2177351 A	08-06-1995
		EP	0734445 A	02-10-1996
		HU	75078 A	28-04-1997
		JP	9511124 T	11-11-1997
		PL	314696 A	16-09-1996
		ZA	9409385 A	27-05-1996
WO 9619588 A	27-06-1996	NONE		
EP 0771879 A	07-05-1997	WO	9534672 A	21-12-1995
WO 8911789 A	14-12-1989	US	5258300 A	02-11-1993
		AU	3540289 A	05-01-1990
		CA	1338349 A	28-05-1996
		CN	1038465 A	03-01-1990
		DE	68912783 D	10-03-1994
		DE	68912783 T	19-05-1994
		EP	0429458 A	05-06-1991
		HU	214630 B	28-08-1998
		JP	3504798 T	24-10-1991

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:
SAVERIO CARL FALCO ET AL.

CASE NO.: BB1037-F

APPLICATION NO.: 09/049,304

GROUP ART UNIT: 1638

FILED: MARCH 27, 1998

EXAMINER: MCELWAIN, E. F.

FOR: CHIMERIC GENES AND METHODS FOR INCREASING THE LYSINE CONTENT OF THE SEEDS OF PLANTS

CONFIRMATION NO.: 5349

Response Pursuant to 37 C.F.R. § 1.111

Commissioner of Patents and Trademarks
Washington, DC 20231

Sir:

This is submitted in response to the Office Action dated August 9, 2002 concerning the above-identified application. Applicants respectfully request reconsideration and submit the following in support thereof.

IN THE CLAIMS:

Please amend the claims as follows; a marked-up version showing changes made is attached hereto:

Please cancel claims 2-3 without prejudice to or disclaimer of the subject matter recited therein.

1. (once amended) An isolated nucleic acid fragment comprising a nucleic acid sequence encoding all or a functional subsequence of a plant lysine ketoglutarate reductase/saccharopine dehydrogenase.

4. (once amended) The nucleic acid fragment of Claim 1 comprising a nucleic acid sequence of SEQ ID NO:120.

5. (once amended) The nucleic acid fragment of Claim 1 wherein the nucleic acid sequence encodes a polypeptide as set forth in SEQ ID NO:122.

6. (once amended) A chimeric gene comprising the isolated nucleic acid fragment of Claim 1 encoding lysine ketoglutarate reductase or a functional subsequence thereof, operably linked to suitable seed-specific regulatory sequences wherein a plant transformed with said chimeric gene has seeds with increase lysine content compared to seeds obtained from untransformed plants.

7. (once amended) The chimeric gene according to Claim 6 wherein the isolated nucleic acid fragment comprises a nucleic acid sequence or functional subsequence of the nucleic acid sequence set forth in SEQ ID NO:120.

11. (once amended) A plant seed transformed with the chimeric gene of claim 6 or 7 wherein said transformed plant seed has an increased lysine content compared to seed obtained from an untransformed plant.

14. (once amended) A method for [reducing lysine ketoglutarate reductase activity] increasing lysine content in a plant seed which comprises:

(a) transforming plant cells with the chimeric gene of claim 6 or 7;

(b) regenerating fertile mature plants from the transformed plant cells obtained from step (a) under conditions suitable to obtain seeds;

(c) screening progeny seed of step (b) for increased lysine content; and

(d) selecting those lines whose seeds have increased lysine content.

Kindly add the following new claims:

--21. (new) An isolated nucleic acid fragment comprising a nucleic acid sequence which is useful in antisense inhibition or sense suppression of endogenous lysine ketoglutarate reductase activity in a transformed corn plant wherein said isolated nucleic acid fragment comprises all or part of the nucleic acid sequence of SEQ ID NO:120.

22. (new) A chimeric gene capable of causing an increased level of lysine in seeds obtained from a transformed corn plant, the chimeric gene comprising a nucleic acid fragment of Claim 21, said fragment being operably linked to at least one regulatory sequence.

23. (new) A corn plant comprising the chimeric gene of claim 22 in its genome.

24. (new) Seed obtained from the corns plant of claim 23.

25. (new) A method for increasing lysine content in a corn plant seed which comprises:

(a) transforming corn plant cells with the chimeric gene of claim 21;

(b) regenerating fertile mature corn plants from the transformed corn plant cells obtained from step (a) under conditions suitable to obtain seeds;

(c) screening progeny seed of step (b) for increased lysine content; and

(d) selecting those lines whose seeds have increased lysine content.

26. (new) Corn olant seed obtained by the method of claim 26 or 27.--

IN THE SPECIFICATION:

Please amend the specification as follows; a marked-up version showing changes made is attached hereto:

Please delete the sequence listing appearing on the bottom of page 7 starting with SEQ ID NOS: 102 and 103 through SEQ ID NO:132 on page 9 and replace with the following:

SEQ ID NOS:102 and 103 are partial cDNAs for plant lysine ketoglutarate reductase/saccharopine dehydrogenase from Arabidopsis thaliana.

SEQ ID NOS:104 and 105 are polypeptides encoded by SEQ ID NOS:102 and 103, respectively that are homologous to fungal saccharopine dehydrogenase (glutamate-forming).

SEQ ID NOS:106 and 107 were used in Example 25 as PCR primers to add Nco I and Kpn I sites at the 5' and 3' ends of the corn DHDPS gene.

SEQ ID NOS:108 and 109 were used for PCR amplification of a 2.24 kb DNA fragment from genomic *Arabidopsis* DNA.

SEQ ID NO:110 shows the sequence of the Arabidopsis lysine ketoglutarate reductase/saccharopine dehydrogenase genomic DNA fragment.

SEQ ID NO:111 shows the sequence of a full length cDNA for plant lysine ketoglutarate reductase/saccharopine dehydrogenase from Arabidopsis thaliana.

SEQ ID NO:112 shows the deduced amino acid sequence of *Arabidopsis* lysine ketoglutarate reductase/saccharopine dehydrogenase protein.

SEQ ID NOS:113 and 114 were used for PCR amplification of soybean and corn lysine ketoglutarate reductase/saccharopine dehydrogenase cDNA fragment.

SEQ ID NO:115 shows the sequence of a soybean lysine ketoglutarate reductase/saccharopine dehydrogenase cDNA fragment.

SEQ ID NO:116 shows the sequence of a corn lysine ketoglutarate reductase/saccharopine dehydrogenase cDNA fragment.

SEQ ID NO:117 shows the partial amino acid sequence of soybean lysine ketoglutarate reductase/saccharopine dehydrogenase protein deduced from SEQ ID NO:115.

SEQ ID NO:118 shows the partial amino acid sequence of corn lysine ketoglutarate reductase/saccharopine dehydrogenase protein deduced from SEQ ID NO:116.

SEQ ID NO:119 shows the sequence of a 2582 nucleotide partial cDNA from soybean for a lysine ketoglutarate reductase/saccharopine dehydrogenase protein.

SEQ ID NO:120 shows the sequence of a 3265 nucleotide partial cDNA from corn for a lysine ketoglutarate reductase/saccharopine dehydrogenase protein.

SEQ ID NO:121 shows the deduced partial amino acid sequence of soybean lysine ketoglutarate reductase/saccharopine dehydrogenase protein encoded by nucleotides 3 through 2357 of SEQ ID NO:119.

SEQ ID NO:122 shows the deduced partial amino acid sequence of corn lysine ketoglutarate reductase/saccharopine dehydrogenase protein encoded by nucleotides 3 through 3071 of SEQ ID NO:120.

SEQ ID NO:123 is a nucleotide sequence corresponding to nucleotides 1 through 1908 of SED ID NO:120.

SEQ ID NO:124 is the deduced amino acid sequence from SEQ ID NO:123.

SEQ ID NO:125 shows the sequence of a 720 nucleotide lysine ketoglutarate reductase/saccharopine dehydrogenase cDNA from rice.

SEQ ID NO:126 shows the deduced partial amino acid sequence of rice lysine ketoglutarate reductase/saccharopine dehydrogenase protein encoded by nucleotides 2 through 720 of SEQ ID NO:125.

SEQ ID NO:127 shows the sequence of a 308 nucleotide lysine ketoglutarate reductase/saccharopine dehydrogenase cDNA from rice.

SEQ ID NO:128 shows the deduced partial amino acid sequence of rice lysine ketoglutarate reductase/saccharopine dehydrogenase protein encoded by nucleotides 1 through 129 of SEQ ID NO:127.

SEQ ID NO:129 shows the sequence of a 429 nucleotide cDNA from wheat.

SEQ ID NO:130 shows the deduced partial amino acid sequence of wheat lysine ketoglutarate reductase/saccharopine dehydrogenase protein encoded by nucleotides 1 through 252 of SEQ ID NO:129.

SEQ ID NO:131 shows the SDH coding region of the *Arabidopsis* cDNA clone.

SEQ ID NO:132 shows the amino acid sequence of the saccharopine dehydrogenase domain of the Arabidopsis lysine ketoglutarate reductase/saccharopine dehydrogenase protein.

REMARKS

Claims 1-20 were pending in the application and Claims 8, 10 and 16-20 were withdrawn. Thus, Claims 1-7, 9 and 11-15 are currently pending.

Claims 1, 2, 3, 6 and 7, and Claims 9 and 11-15 dependent thereon, were rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

It is stated on page 2 of the Office Action that Claim 1 is indefinite in the recitation of "or part" with regard to the nucleic acid sequence, since a part can be as little as one nucleotide. Applicants have amended Claim 1 to replace "part" with "a functional subsequence". A functional subsequence will not be as little as one nucleotide. Support for this amendment can be found at least on page 11 in the definition of the term "essentially similar" and on page 9, line 26. Claim 1 was also amended by adding the term "plant" before lysine ketoglutarate/saccharopine dehydrogenase. Support for this amendment can be found in the specification on page 31. Thus, no new matter has been added.

It is stated on page 2 of the Office Action that Claims 2, 3 and 7 are indefinite in the recitation of "essentially similar" given that it is unclear what the metes and bounds of this term would be regarding the claimed sequences. Claim 2 has been cancelled in view of Claim 5 and Claim 3 has been cancelled in view of Claim 4. Applicants have amended Claim 7 to recite a subsequence of the nucleic acid sequence set forth in SEQ ID NO:120. Thus, no new matter has been added.

It is stated on page 2 of the Office Action that Claim 6 is indefinite in the recitation of "subfragment thereof" with regard to the LKR gene, since a subfragment can be as little as one nucleotide. Claim 6 has been amended to replace the term "subfragment" with "functional subsequence". Once again, a functional subsequence will not be as little as one nucleotide and the Examiner's attention is kindly invited to page 9, line 26 of the specification which discusses the terms subfragment and subsequence. In addition, the claim has been reworded to clarify that seeds obtained from a plant transformed with the chimeric gene have reduced reductase activity when compared to seeds obtained from an untransformed plant. Accordingly, no new matter has been added.

In view of the amendments to Claims 1, 6 and 7, withdrawal of the rejections of these claims pursuant to 35 U.S.C. 112, first paragraph, is respectfully requested.

Claims 6, 11 and 14 were amended to recite seeds having an increased lysine content. Support for this can be found on page 31, last three lines, and in Example 23, specifically, Table 17. Thus, no new matter has been added.

In addition, claims 21-26 have been added which address blocking expressin of the LKR/SDH gene in transformed plants using antisense or cosuppression. This is discussed in the specification on page 34, last two lines through line 3 on page 36 and in the Examples. Thus, no new matter has been added.

Claims 1-3, 6, 7, 9 and 11-15 were rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

It is stated on page 4 of the Office Action that the specification fails to describe structural features that are essential for LKR activity. The Examiner's attention is kindly invited to pages 31-35 and Example 20 on pages 92-98 of the specification which describe the isolation of an *Arabidopsis* and corn lysine ketoglutarate reductase/saccharopine dehydrogenase (LKR/SDH) gene.

The discussion on pages 31-35 indicates that:

> The deduced amino acid sequence of *Arabidopsis* LKR/SDH protein is shown in SEQ ID NO:112. The amino acid sequence shows that in plants LKR and SDH enzyme activities are carried on a single bi-functional protein, and that the protein lacks an N-terminal targeting sequence indicating that the lysine degradative pathway is located in the plant cell cytosol. The amino acid sequence of *Arabidopsis* LKR/SDH protein was compared to that of other LKR and SDH proteins thus revealing regions of conserved amino acid sequence. Degenerate oligonucleotides can be designed based upon this information and used to amplify genomic or cDNA fragments via PCR from other organisms, preferably plants. As an example of this, SEQ ID NO:113 and SEQ ID NO:114 were designed and used to amplify soybean and corn LKR/SDH cDNA fragments. The sequence of a partial soybean LKR/SDH cDNA is shown in SEQ ID NO:115, and the sequence of a partial corn cDNA is shown in SEQ ID NO:116. These DNA fragments can be used to isolate larger genomic DNA fragments, which include the entire coding region, as well as 5' and 3' flanking regions, via hybridization to corn or soybean genomic DNA or cDNA libraries, as was done for *Arabidopsis*. More complete sequence information from the coding regions for soybean and corn LKR/SDH was obtained using the sequences in SEQ ID NOS:115 and 116 as starting materials in protocols such as 5' RACE and hybridization to cDNA libraries. A near full-length cDNA for soybean LKR/SDH is shown in SEQ ID NO:119, and a near full-length cDNA for corn LKR/SDH is shown in SEQ ID NO:120. A truncated version of the LKR/SDH cDNA from corn is set forth in SEQ ID NO:123.
>
> The deduced partial amino acid sequences of soybean LRK/SDH protein is shown in SEQ ID NOS:117 and 121 and the deduced partial amino acid sequences of corn LKR/SDH protein is shown in SEQ ID NO:118, 122 and 124. These amino acid sequences can be compared to other LKR/SDH protein sequences, e.g., the *Arabidopsis* LKR/SDH protein sequence, thus revealing regions of conserved amino acid sequence. With this information oligonucleotide primers can be designed and synthesized to permit isolation of LKR/SDH genomic or cDNA fragments from any plant source.

In addition, submitted herewith is a copy of Epelbaum et al., *Plant Mol. Biol.* 35:735-748 (1997) entitled "Lysine-ketoglutarate reductase and saccharopine

dehydrogenase from *Arabidopsis thaliana*: nucleotide sequence and characterization." The authors are also the co-inventors of the above-identified application. It is noted that Applicants were the first to report of the molecular cloning of a plant LKR/SDH genomic and cDNA sequence. The Epelbaum paper describes how LKR and SDH specific activity was determined using previously described assays with some minor modifications. A diagram of the *Arabidopsis* LKR-SDH gene structure is shown in Figure 3 of the Epelbaum paper.

It is stated on page 96 of the specification that:

> The complete genomic sequence of the *Arabidopsis* LKR/SDH gene is shown in SEQ ID NO:110. The sequence includes approximately 2 kb of 5' noncoding sequence and 500 bp of 3' noncoding sequence and 23 introns. Overlapping fragments of the corresponding cDNA were isolated from total *Arabidopsis* RNA by RT-PCR. **Sequence analysis of the LKR-SDH cDNA revealed an ORF of 3.16 kb, which predicts a protein of 117 kd, and confirms that the LKR and SDH enzymes reside on one polypeptide.** The complete protein coding sequence of *Arabidopsis* LKR/SDH gene, derived from the cDNA, is shown in SEQ ID NO:111. The deduced amino acid sequence of *Arabidopsis* LKR/SDH protein is shown in SEQ ID NO:112. The protein lacks an N-terminal targeting sequence implying that the lysine degradative pathway is located in the plant cell cytosol.
>
> Degenerate oligonucleotides, SEQ ID NO:113 and SEQ ID NO:114, were designed based upon comparison of the *Arabidopsis* LKR/SDH amino acid sequence with that of other LKR proteins. These were used to amplify soybean and corn LKR/SDH cDNA fragments using PCR from mRNA, or cDNA synthesized from mRNA, isolated from developing soybean or corn seeds. The soybean and corn PCR-generated cDNA fragments were cloned and sequenced. The sequence of the soybean LKR/SDH cDNA fragment is shown in SEQ ID NO:115, and the sequence of the corn cDNA fragment is shown in SEQ ID NO:116. The deduced partial amino acid sequence of soybean LKR/SDH protein is shown in SEQ ID NO:117 and the deduced partial amino acid sequence of corn LKR/SDH protein is shown in SEQ ID NO:118. The partial cDNAs encoding corn and soybean LKR/SDH obtained by PCR, above, were used in protocols that extended the sequence information for these functions. These protocols, which included RACE and direct DNA:DNA hybridization to cDNA libraries for the identification of overlapping clones, are well known to persons skilled in the art. From these efforts, more complete sequences for the corn and soybean cDNAs for LKR/SDH were obtained. **SEQ ID NOS:119 and 120 list, respectively, near full-length sequences for the LKR/SDH coding regions from soybean and corn. The deduced protein sequences encoded by these soybean and corn cDNAs are shown in SEQ ID NOS:121 and 122, respectively.** (Emphasis added.)

Other references that demonstrate that the nucleotide sequences described in the invention encode plant LKR and SDH proteins are Tang et al., *Plant Cell* 9:1305-1316 (1997) entitled "Regulation of lysine catabolism through lysine-ketoglutarate reductase and saccharopine dehydrogenase in *Arabidopsis*" (discussed below) and Kemper et al., *Eur. J. Biochem.* 253:720-729 (1998) entitled "Structure and regulation of the bifunctional enzyme lysine-oxoglutarate reductase-saccharopine dehydrogenase in maize" (both articles are submitted herewith). Kemper et al. do not disclose the nucleotide or amino acid sequence of their bifunctional corn LKR/SDH, but they do use limited proteolysis to assess the structure/function of this enzyme.

It is respectfully submitted that the foreoing clearly demonstrates a correlation between structure and function. Thus, it is respectfully submitted in view of the above discussion and information and given the teachings in the art regarding the correlation between structure and function one skilled in the art would conclude that the inventors were in possession of the claimed invention at the time the application was filed. Accordingly, withdrawal of the rejections pursuant to 35 U.S.C. 112, first paragraph, is respectfully requested.

Claims 1-7, 9 and 11-15 were rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

It is stated on page 4 of the Office Action that "the specification does not demonstrate that any of the claimed sequences encode a protein having LKR activity."

Submitted herewith is a copy of Tang et al., The Plant Cell, Vol. 9, 1305-1316 (August 1997) which is entitled "Regulation of Lysine Catabolism through Lysine-Ketoglutarate Reductase and Saccharopine Dehydrogenase in Arabidopsis". This paper reports the cloning of an Arabidopsis cDNA encoding a bifunctional polypeptide that contains both of these enzyme activities linked to each other.

The Arabidopsis sequence disclosed in this paper is identical to SEQ ID NO:111 that has been disclosed in Applicant's earlier filed cases of which priority is claimed.

It is stated on page 1308, right-hand column, of Tang et al. that:

> To determine further whether the N-terminal part of cAt-LKR/SDH encodes an LKR enzyme, the entire coding sequence of this cDNA was subcloned into the bacterial expression vector pET-15b and used to transform *E. coli* cell.s Bacterial cells harboring this plasmid had SDH but no LKR activity (data not shown). Because bacterial cells did not produce an active LKR we attempted to express the *Arabidopsis* LKR protein in yeast cells. Yeast has a monofunctional LKR enzyme, so we subcloned the N terminus of the presumed LKR domain of Cat-LKR/SDH into the yeast expression vector pVT-102u and transformed this plasmid into the yeast Lys 1 mutant. **As shown in Figure 9, yeast cells harboring this plasmid have significantly higher LKR activity than do control cells transformed with the same plasmid without the LKR insert, thereby confirming our supposition that cAT-LKR/SDH indeed encodes a bifunctional LKR/SDH enzyme.** (Emphasis added).

It is noted that on page 35 of the specification, last full paragraph, it is stated that "High level expression of *Arabidopsis* SDH was achieved in *E. coli* and the SDH protein has been purified from the bacterial extracts, and used to raise rabbit antibodies to the protein." Given, that the Arabidopsis sequence (SEQ ID NO:111) disclosed in the instant application is identical to that described by Tang et al., it

would be expected that this sequence would produce LKR activity if expressed in yeast as described by Tang et al.

Structural and Functional properties of the bifunctional LKR/SDH enzyme are discussed in the Tang et al. paper on starting on page 1312, left hand column.

Analysis of LKR and SDH activities is described on page 1315, left hand column, and it should be clear to those skilled in the art that such analysis would not require any undue experimentation.

Submitted herewith is a copy of a Declaration of Dr. Carl Falco, one of the co-inventors of the subject case, (the original version can be found in the file of Application No. 08/823,771 (Attorney Docket No. BB-1037-D)). Please note that priority of the '771 application was claimed. Dr. Falco's Declaration dated August 24, 2000, shows that with the *Arabidopsis* LKR/SDH fragments in hand, it was possible to isolate LKR/SDH fragments from any other plant desired, and use them to block expression utilizing antisense inhibition and/or cosuppression. Dr. Falco's Declaration demonstrated that blocking the first step in lysine catabolism, i.e., "knocking out" LKR/SDH, leads to increased accumulation of lysine in seeds. It is stated specifically in paragraph 9 of Dr. Falco's August 24, 2000 declaration that :

> 9. The corn LKR/SDH cDNA sequence was used to identify transposon mutations in the endogenous corn LKR/SDH gene via PCR screening of a library of corn lines containing Robertson's Mutator transposon insertions. The precise location of Mutator insertions into the LKR/SDH gene was determined by sequencing of genomic DNA from individual mutants. An insertion mutation located in an exon in the LKR domain of the gene was chosen for further study. Southern blot analysis of corn genomic DNA indicated that corn contains only one LKR/SDH gene. Since an insertion mutation is expected to block function of the gene, it was anticipated that such a mutation would be recessive. One fourth of the progeny seed from a selfed corn ear with such a mutation segregating would be expected to be homozygous for the mutation. It was observed that approximately one fourth of such seed exhibited a higher level of free lysine than normal (5 to 15 fold higher) without the increase in the lysine catabolite saccharopine that is seen when free lysine is increased via expression of lysine insensitive DHDPS. It was concluded that knocking out LKR/SDH, by itself, was able to increase seed lysine content in corn seeds.
>
> The LKR/SDH Mutator insertion line was crossed by a transgenic line that accumulates excess free lysine due to expression of lysine insensitive DHDPS and AK. In this cross two genetic loci that affect lysine accumulation, one of which is recessive (the LKR/SDH Mutator insertion) and one of which is semi-dominant (the lysine insensitive DHDPS and AK trangene locus), are segregating. Single seeds were analyzed for lysine and saccharopine content. The most striking observation from this experiment is that the highest lysine containing seeds have low levels of saccharopine (see figure). The low saccharopine level indicates that these seeds are homozygous for the LKR/SDH Mutator insertion, while the high lysine level indicates that they carry the lysine insensitive DHDPS and AK trangene locus. The level of lysine accumulation is considerably higher (2-3 fold) than the level provided by the DHDPS and AK trangene locus alone. Thus, this experiment demonstrates that an increase in the accumulation of lysine, accompanied by a reduction in accumulation of lysine catabolites can be accomplished by combination of lysine overproduction brought about by expression of lysine insensitive DHDPS + AK and reduction of lysine catabolism by blocking expression of LKR/SDH, as we taught in the patent application. These results show that the concern stated in the Office Action on page 5 that

"modifying metabolic pathways ... is highly unpredictable and often the desirable results are impossible to achieve" is unfounded in this particular case.

As indicated above, LKR/SDH expression has been blocked in corn via cosuppression. To accomplish this a chimeric gene designed for cosuppression of LKR was constructed by linking a 1268 bp LKR/SDH gene fragment, which included the LKR coding domain, to the corn endosperm 27 kD zein promoter and 10 kD zein 3' untranslated region. This chimeric gene was introduced into corn by particle-gun mediated transformation. Over 100 transformed lines were obtained. Of 72 transformation events that were regenerated into plants and produced seed, 13 had seeds with a greater than four fold increase in free lysine. This is a typical frequency for cosuppression events. Since the transformed plants were out-crossed, the transgenic locus must be dominant or there would not have been any observable phenotype. This is expected from a cosuppression transgene, and is an advantage over knock-out mutations like the LKR/SDH Mutator insertion described above.

Some of the LKR cosuppression transformants have been carried forward for further testing. An event that has continued to show the increased free lysine phenotype for several generations and behaves genetically as a single locus transgene insertion has been selected for crossing to the transgenic line that accumulates excess free lysine due to expression of lysine insensitive DHDPS and AK. Results from that experiment are not yet available, but the expectation is that seeds carrying both transgene loci will have higher lysine levels than either parent, as was observed in the LKR Mutator insertion cross described above. In addition, co-transformation experiments in which the chimeric gene designed for cosuppression of LKR described above has been combined with a chimeric gene for expression of lysine insensitive DHDPS and introduced into corn by particle-gun mediated transformation are in progress. This is expected to yield transformants that produce seeds with the high lysine level observed in the LKR Mutator insertion cross by lysine insensitive DHDPS and AK, but with both chimeric genes at a single genetic locus, which is highly desirable for corn breeding.

Also submitted herewith is a copy of a second Declaration of Dr. Carl Falco, (the original version can be found in the file of Application No. 08/823,771 (Attorney Docket No. BB-1037-D)). Once again, please note that priority of the '771 application was claimed. Dr. Falco's Declaration dated February 16, 2001, sets forth data showing seeds with increased lysine that were obtained from plants co-transformed with DHDPS and LKR.

The experiments discussed in Dr. Falco's Declaration dated February 16, 2001 along with the information submitted herewith and the detailed description of the invention provided in the instant application demonstrate that seeds having an increased lysine content can be made when a lysine insensitive DHDPS gene (with or without a lysine insensitive AK gene) is combined with a co-suppressing LKR gene.

It is stated on page 4 of the Office Action that "It is well established that sequence similarity is not sufficient to determine functionality of a cDNA coding sequence."

Applicants respectfully submit that in view of Tang et al. it is clear that there is a correlation between sequence similarity and functionality insofar as LKR/SDH activity is concerned.

Thus, it is respectfully submitted in view of the above discussion and references, that no undue experimentation would be needed to practice the claimed invention. Accordingly, withdrawal of the rejections pursuant to 35 U.S.C. 112, first paragraph, is requested.

It is respectfully submitted that the application is in form for allowance which allowance is respectfully requested.

A petition for a two (2) month extension of time, a version with markings to show changes made and copies of the above-identified documents accompany this response.

The Commissioner is authorized to charge Deposit Account No. 04-1928 (E. I. du Pont de Nemours and Company) for any fees associated with the filing of this response.

Respectfully submitted,

Lynne M. Christenbury

LYNNE M. CHRISTENBURY
ATTORNEY FOR APPLICANTS
REGISTRATION NO. 30,971
TELEPHONE: 302-992-5481
FACSIMILE: 302-892-1026

Dated: Dec. 17, 2002

VERSION WITH MARKING TO SHOW CHANGES MADE

In showing the changes, deleted material is shown in bolded brackets and stricken through, and inserted material is shown underlined.

IN THE CLAIMS:

1. (once amended) An isolated nucleic acid fragment comprising a nucleic acid sequence encoding all or [part] a functional subsequence of a plant [lysine ketoglutarate reductase] lysine ketoglutarate reductase/saccharopine dehydrogenase.

4. (once amended) The nucleic acid fragment of Claim 1 comprising a nucleic acid sequence of [SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:115, SEQ ID NO:116, SEQ ID NO:119,] SEQ ID NO:120[, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129 or SEQ ID NO:131].

5. The nucleic acid fragment of Claim 1 wherein the nucleic acid sequence encodes a polypeptide as set forth in [SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:112, SEQ ID NO:117, SEQ ID NO:118, SEQ ID NO:121,] SEQ ID NO:122[, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:130 or SEQ ID NO:132].

6. (once amended) A chimeric gene comprising the isolated nucleic acid fragment of Claim 1 encoding lysine ketoglutarate reductase or a [subfragment] functional subsequence thereof, operably linked to suitable seed-specific regulatory sequences wherein a plant transformed with said chimeric gene has seeds with [reduced slysine ketoglutarate reductase activity] increased lysine content compared to [in] seeds obtained from untransformed [of] plants [transformed with the chimeric gene].

7. (once amended) The chimeric gene according to Claim 6 wherein the isolated nucleic acid fragment comprises a nucleic acid sequence or functional subsequence thereof [essentially similar to that of] as set forth in [SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:115, SEQ ID NO:116, SEQ ID NO:119,] SEQ ID NO:120[, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129 or SEQ ID NO:131].

11. (once amended) A plant seed transformed with the chimeric gene of Claim 6 or 7 wherein said transformed plant seed has [reduced lysine ketoglutarate reductase activity] an increased lysine content compared to seed obtained from an untransformed plant.

14. (once amended) A method for [reducing lysine ketoglutarate reductase activity] increasing lysine content in a plant seed which comprises:

(a) transforming plant cells with the chimeric gene of claim 6 or 7;

(b) regenerating fertile mature plants from the transformed plant cells obtained from step (a) under conditions suitable to obtain seeds;

(c) screening progeny seed of step (b) for [reduced lysine ketoglutarate reductase activity] increased lysine content; and

(d) selecting those lines whose seeds [contain reduced lysine ketoglutarate reductase activity] have increased lysine content.

Kindly add the following new claims:

21. (new) An isolated nucleic acid fragment comprising a nucleic acid sequence which is useful in antisense inhibition or sense suppression of endogenous lysine ketoglutarate reductase activity in a transformed plant wherein said isolated nucleic acid fragment comprises all or part of the nucleic acid sequence of SEQ ID NO:120.

22. (new) A chimeric gene capable of causing an increased level of lysine in seeds obtained from a transformed plant, the chimeric gene comprising a nucleic acid fragment of Claim 21, said fragment being operably linked to at least one regulatory sequence.

23. (new) Plants comprising the chimeric gene of claim 22 in their genome.

24. (new) Seeds obtained from the plants of claim 23.

25. (new) The plants of claim 23 wherein said plants are selected from the group of plants consisting of *Arabidopsis*, corn, soybean, rapeseed, wheat and rice.

26. (new) A method for increasing lysine content in a plant seed which comprises:

(a) transforming plant cells with the chimeric gene of claim 22;

(b) regenerating fertile mature plants from the transformed plant cells obtained from step (a) under conditions suitable to obtain seeds;

(c) screening progeny seed of step (b) for increased lysine content; and

(d) selecting those lines whose seeds have increased lysine content.

27. (new) The method of claim 27 wherein said plant cell is selected from the group of plants consisting of *Arabidopsis*, corn, soybean, rapeseed, wheat and rice.

28. (new) Plant seed obtained by the method of claim 26 or 27.

IN THE SPECIFICATION:

Please delete the sequence listing appearing on page 8 in its entirety and on page 9 through SEQ ID NO:132 with the following:

SEQ ID NOS:102 and 103 are partial cDNAs for plant lysine ketoglutarate reductase/saccharopine dehydrogenase from *Arabidopsis thaliana*.

SEQ ID NOS:104 and 105 are polypeptides encoded by SEQ ID NOS:102 and 103, respectively that are homologous to fungal saccharopine dehydrogenase (glutamate-forming) [encoded by SEQ ID NOS:102 and 103, respectively].

SEQ ID NOS:106 and 107 were used in Example 25 as PCR primers to add Nco I and Kpn I sites at the 5' and 3' ends of the corn DHDPS gene.

SEQ ID NOS:108 and 109 were used for PCR amplification of a 2.24 kb DNA fragment from genomic *Arabidopsis* DNA.

SEQ ID NO:110 shows the sequence of the *Arabidopsis* [LKR/SDH] lysine ketoglutarate reductase/saccharopine dehydrogenase genomic DNA fragment.

SEQ ID NO:111 shows the [sequence of the Arabidopsis LKR/SDH cDNA] sequence of a full length cDNA[s] for plant lysine ketoglutarate reductase/saccharopine dehydrogenase from *Arabidopsis thaliana*.

SEQ ID NO:112 shows the deduced amino acid sequence of *Arabidopsis* [LKR/SDH] lysine ketoglutarate reductase/saccharopine dehydrogenase protein.

SEQ ID NOS:113 and 114 were used for PCR amplification of soybean and corn [LKR/SDH] lysine ketoglutarate reductase/saccharopine dehydrogenase cDNA fragment.

SEQ ID NO:115 shows the sequence of a soybean [LKR/SDH] lysine ketoglutarate reductase/saccharopine dehydrogenase cDNA fragment.

SEQ ID NO:116 shows the sequence of a corn [LKR/SDH] lysine ketoglutarate reductase/saccharopine dehydrogenase cDNA fragment.

SEQ ID NO:117 shows the [deduced] partial amino acid sequence of soybean [LKR/SDH] lysine ketoglutarate reductase/saccharopine dehydrogenase protein deduced from SEQ ID NO:115.

SEQ ID NO:118 shows the [deduced] partial amino acid sequence of corn [LRK/SDH] lysine ketoglutarate reductase/saccharopine dehydrogenase protein deduced from SEQ ID NO:116.

SEQ ID NO:119 shows the sequence of a 2582 nucleotide partial cDNA from soybean for a lysine ketoglutarate reductase/saccharopine dehydrogenase protein.

SEQ ID NO:120 shows the sequence of a 3265 nucleotide partial cDNA from corn for a lysine ketoglutarate reductase/saccharopine dehydrogenase protein.

.SEQ ID NO:121 shows the deduced partial amino acid sequence of soybean [LKR/SDH] lysine ketoglutarate reductase/saccharopine dehydrogenase protein encoded by nucleotides 3 through 2357 of SEQ ID NO:119.

SEQ ID NO:122 shows the deduced partial amino acid sequence of [soybean] corn [LKR/SDH] lysine ketoglutarate reductase/saccharopine dehydrogenase protein encoded by nucleotides 3 through 3071 of SEQ ID NO:120.

SEQ ID NO:123 is a nucleotide sequence corresponding to nucleotides 1 through 1908 of SED ID NO:120.

SEQ ID NO:124 is the deduced amino acid sequence from SEQ ID NO:123.

SEQ ID NO:125 shows the sequence of a 720 nucleotide [LKR/SDH] lysine ketoglutarate reductase/saccharopine dehydrogenase cDNA from rice.

SEQ ID NO:126 shows the deduced partial amino acid sequence of rice [LKR/SDH] lysine ketoglutarate reductase/saccharopine dehydrogenase protein encoded by nucleotides 2 through 720 of SEQ ID NO:125.

SEQ ID NO:127 shows the sequence of a 308 nucleotide [LKR/SDH] lysine ketoglutarate reductase/saccharopine dehydrogenase cDNA from rice.

SEQ ID NO:128 shows the deduced partial amino acid sequence of rice [LKR/SDH] lysine ketoglutarate reductase/saccharopine dehydrogenase protein encoded by nucleotides 1 through 129 of SEQ ID NO:127.

SEQ ID NO:129 shows the sequence of a 429 nucleotide cDNA from wheat.

SEQ ID NO:130 shows the deduced partial amino acid sequence of wheat [LKR/SDH] lysine ketoglutarate reductase/saccharopine dehydrogenase protein encoded by nucleotides 1 through 252 of SEQ ID NO:129.

SEQ ID NO:131 shows the SDH coding region of the *Arabidopsis* cDNA clone.

SEQ ID NO:132 shows the amino acid sequence of the [SDH] saccharopine dehydrogenase[.] domain of the *Arabidopsis* [LKR/SDH] lysine ketoglutarate reductase/saccharopine dehydrogenase protein.

PTO/SB/22(10-00)
Approved for use through 10/31/2002. OMB 0651-0031
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a)	Docket Number (Optional) BB1037-F

In re Application of Saverio Carl Falco	
Application Number 09/049,304	Filed March 27, 1998
For Chimeric Genes and Methods for Increasing the Lysine Content of the Seeds of Plants	
Group Art Unit 1638	Examiner E. F. McElwain

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above identified application.

The requested extension and appropriate non-small-entity fee are as follows (check time period desired):

☐	One month (37 CFR 1.17(a)(1))	$
☒	Two months (37 CFR 1.17(a)(2))	$400.00
☐	Three months (37 CFR 1.17(a)(3))	$
☐	Four months (37 CFR 1.17(a)(4))	$
☐	Five months (37 CFR 1.17(a)(5))	$

☐ Applicant claims small entity status. See 37 CFR 1.27. Therefore, the fee amount shown above is reduced by one-half, and the resulting fee is: $ _____ .

☐ A check in the amount of the fee is enclosed.

☐ Payment by credit card. Form PTO-2038 is attached.

☐ The Commissioner has already been authorized to charge fees in this application to a Deposit Account.

☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 04-1928 .
I have enclosed a duplicate copy of this sheet.

I am the ☐ applicant/inventor.

☐ assignee of record of the entire interest. See 37 CFR 3.71
Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96).

☒ attorney or agent of record.

☐ attorney or agent under 37 CFR 1.34(a).
Registration number if acting under 37 CFR 1.34(a). _____ .

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Dec. 17, 2002
Date

Lynne M. Christenbury
Signature

Lynne M. Christenbury
Typed or printed name

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below*.

☒ *Total of 1 forms are submitted.

PTO/SB/17 (10-02)
Approved for use through 10/31/2002. OMB 0651-0032
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE
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FEE TRANSMITTAL for FY 2003

Patent fees are subject to annual revision.

☐ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 400.00

Complete if Known	
Application Number	09/049,304
Filing Date	March 27, 1998
First Named Inventor	Saverio Carl Falco et al.
Examiner Name	E. F. McElwain
Group / Art Unit	1638
Attorney Docket No.	BB1037-F

METHOD OF PAYMENT (check all that apply)

☐ Check ☐ Credit card ☐ Money Order ☐ Other ☐ None

☒ Deposit Account:

Deposit Account Number: 04-1928

Deposit Account Name: E. I. du Pont de Nemours and Company

The Commissioner is authorized to: *(check all that apply)*
☒ Charge fee(s) indicated below ☒ Credit any overpayments
☒ Charge any additional fee(s) during the pendency of this application
☐ Charge fee(s) indicated below, **except for the filing fee to the above-identified deposit account.**

FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1001	740	2001	370	Utility filing fee	
1002	330	2002	165	Design filing fee	
1003	510	2003	255	Plant filing fee	
1004	740	2004	370	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	

SUBTOTAL (1) ($) 0.00

2. EXTRA CLAIM FEES

			Extra Claims		Fee from below	=	Fee Paid
Total Claims		-20 ** =	0	X	18	=	0
Independent Claims		-3 ** =	0	X	84	=	0
Multiple Dependent	☐			X	280	=	

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	84	2201	42	Independent claims in excess of 3
1203	280	2203	140	Multiple dependent claim, if not paid
1204	84	2204	42	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($) 0.00

**or number previously paid, if greater; For Reissues, see above

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet.	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	400	2252	200	Extension for reply within second month	400
1253	920	2253	460	Extension for reply within third month	
1254	1,440	2254	720	Extension for reply within fourth month	
1255	1,960	2255	980	Extension for reply within fifth month	
1401	320	2401	160	Notice of Appeal	
1402	320	2402	160	Filing a brief in support of an appeal	
1403	280	2403	140	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive – unavoidable	
1453	1,280	2453	640	Petition to revive – unintentional	
1501	1,280	2501	640	Utility issue fee (or reissue)	
1502	460	2502	230	Design issue fee	
1503	620	2503	310	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17 (q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	740	2809	370	Filing a submission after final rejection (37 CFR § 1.129(a))	
1810	740	2810	370	For each additional invention to be examined (37 CFR § 1.129(b))	
1801	740	2801	370	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	

Other fee (specify)

*Reduced by Basic Filing Fee Paid

SUBTOTAL (3) ($) 400.00

SUBMITTED BY

					Complete *(if applicable)*
Name (Print/Type)	Lynne M. Christenbury	*Registration No. (Attorney/Agent)*	30,971	*Telephone*	302-992-5481
Signature	Lynne M. Christenbury			*Date*	Dec. 17, 2002

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

This collection of information is required by 37 CFR 1.17 and 1.27. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducint this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office,

Ug r Case

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN THE APPLICATION OF:

SAVERIO C. FALCO
SHARON J. KEELER
JANET A. RICE

CASE NO.: BB-1037-D

APPLN. NO.: 08/823,771

GROUP ART UNIT: 1638

FILED: MARCH 24, 1997

EXAMINER: E. MCELWAIN

FOR: CHIMERIC GENES AND METHODS FOR INCREASING THE LYSINE AND THREONINE CONTENT OF THE SEEDS OF PLANTS

Date: AUGUST 24, 2000

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

Declaration of Dr. Carl Falco Pursuant to 37 CFR §1.132

I, Saverio Carl Falco, am a citizen of the United States of America, residing at 1902 Miller Road, Arden, Delaware 19810, United States of America, and I declare as follows:

1. I am one of the above-identified inventors named in this application. I am a graduate of Rutgers University of New Brunswick, New Jersey with a B.A. degree granted in 1971 with high honors and distinction in physics. I received a Ph.D. in 1977 from the University of Chicago in biochemistry and molecular biology. From 1977 to 1981 I was a National Institutes of Health postdoctoral fellow at the Massachusetts Institute of Technology. I have been employed by E. I. du Pont de Nemours and Company since 1981 directing and conducting research in plant genetic engineering.

2. I have reviewed the Office Action dated April 25, 2000. I am aware that this declaration is being submitted to address the concerns set forth on page 4 and 5 of the Office Action that "the specification does not disclose any plants that comprise the claimed two gene fragments that result in the claimed increase in lysine relative to a

I HEREBY CERTIFY THAT THIS PAPER IS BEING DEPOSITED WITH THE UNITED STATES POSTAL SERVICE WITH SUFFICIENT POSTAGE AS FIRST CLASS MAIL IN AN ENVELOPE ADDRESSED TO: ASST. COMMISSIONER FOR PATENTS, WASHINGTON, D.C. 20231, ON THIS DATE.

8/24/00
Date

R. Nanette Marcus
R. Nanette Marcus

plant that does not comprise said two gene fragments. In addition, the specification fails to provide guidance with regard to the choice of subfragments that will result in the antisense inhibition or cosuppression of LKR."

3. At the outset, it is noted that many components of the process of plant genetic engineering, e.g. construction of chimeric genes for expression in plant cells, or for blocking expression of endogenous genes, transformation of plants, have become routine for those skilled in the art. Notwithstanding this, what follows is intended to show that one of ordinary skill in the art could follow the teachings of the instant application to practice the claimed invention without engaging in undue experimentation.

4. First, the rationale for combining the nucleic acid fragments of the invention are clearly disclosed in the specification. It was shown, for the first time, that accumulation of excess free lysine in plant seeds, accomplished via expression of lysine insensitive DHDPS, is accompanied by breakdown of free lysine and accumulation of intermediates in the breakdown pathway such as saccharopine. Thus, there was a clear incentive to reduce the loss of excess lysine due to catabolism.

5. Second, methods were provided to prevent lysine catabolism through reduction in the activity of the enzyme lysine ketoglutarate reductase (LKR), which catalyzes the first step in lysine breakdown. This can be accomplished by introducing a mutation in the plant gene that encodes LKR that reduces or eliminates enzyme function. Such mutations can be identified by screening mutants for lysine over-producer lines that do not accumulate the lysine breakdown products, saccharopine and α-amino adipic acid. Alternatively, the first nucleic acid fragments containing plant LKR cDNAs were disclosed. The nucleotide sequences of these fragments make it straightforward to isolate LKR nucleic acid fragments from any plant desired (see point 6 below). Chimeric genes for expression of antisense LKR RNA or for cosuppression of LKR in the seeds of plants can then be created. The chimeric LKR gene can be linked to chimeric genes encoding lysine insensitive AK and DHDPS and all introduced into plants via transformation simultaneously, or the chimeric LKR gene or mutant LKR gene can be brought together with chimeric genes encoding lysine insensitive AK and DHDPS by crossing plants to create hybrids carrying two or more of the genes (see below).

6. Third, examples of all of the nucleic acid fragments of the invention were provided in the specification of the subject case. In the case of the bifunctional protein lysine ketoglutarate reductase (LKR)/saccharopine dehydrogenase (SDH), two plant nucleic acid fragments (SEQ ID NOS:102 and 103) containing cDNA derived

from the plant *Arabidopsis thaliana* were provided in the present patent application. In the application it was stated that full length cDNAs encoding plant LKR plus saccharopine dehydrogenase (SDH) or genomic DNAs containing the entire LKR/SDH gene can be readily identified by hybridization to labelled cDNA fragments of SEQ ID NO:102: or SEQ ID NO:103: and thus isolated. This was, in fact, accomplished and is described in Epelbaum, S., McDevitt, R. and Falco, S. C., (1997) "Lysine-ketoglutarate reductase and saccharopine dehydrogenase from *Arabidopsis thaliana*: nucleotide sequence and characterization", Plant Mol. Biol. 35, 735.

The availability of the Arabidopsis LKR/SDH gene made it straightforward for us, as it would be for anyone skilled in the art, to isolate other plant LKR/SDH genes. Degenerate oligonucleotides were designed based upon highly conserved regions of the deduced amino acid sequence of plant and fungal proteins and used to amplify soybean and corn LKR/SDH cDNA fragments. Near full-length cDNAs for soybean and corn LKR/SDH were then isolated using 5' RACE and hybridization to cDNA libraries. LKR/SDH nucleic acid fragments were isolated from several other plant species including wheat and rice by identifying EST sequences homologous to the already known plant LKR/SDH sequences.

7. Fourth, there is a description of how to use these nucleic acid fragments to practice the invention. In the case of LKR/SDH, the availability of plant LKR/SDH genes made it possible to block expression of the LKR/SDH gene in transformed plants via antisense inhibition or cosuppression. It was stated in the Office Action on page 4 that antisense inhibition and cosuppression of a gene in a plant is unpredictable. This is true only in the sense that every transformant does not produce the desired phenotype. But one skilled in the art is well aware of this and designs the experiment in a way that many transformants are obtained and screened for the desired phenotype.

My own experience with cosuppression methodology in plants, as well as my knowledge of the work of my colleagues, and research work in the broader scientific community, indicates that this method is reliable and predictable. The use of cosuppression to block expression of several different genes in several different plants has been achieved ~~[quite]~~ successfully at DuPont.

Specifically in the case of LKR/SDH, cosuppression has been used to block expression with the first gene fragment and promoter combination tested, which hardly represents undue experimentation (see point 10 below).

8. It is stated on page 5 of the Office Action that "De Luca teaches that modifying metabolic pathways by transforming plants with genes that control steps of the pathway is highly unpredictable and often the desirable results are impossible to achieve." This may be true in cases where not enough is known about the metabolic pathway, but in the case of the lysine biosynthetic and catabolic pathways, it has been demonstrated how to increase production of lysine via modification of the biosynthetic pathway using lysine insensitive DHDPS and AK, and shown that accumulation of free lysine in seeds is also controlled by catabolism of lysine. We teach that blocking the first step in lysine catabolism will lead to increased accumulation of lysine and this is, in fact, what we have observed as described below.

9. The corn LKR/SDH cDNA sequence was used to identify transposon mutations in the endogenous corn LKR/SDH gene via PCR screening of a library of corn lines containing Robertson's Mutator transposon insertions. The precise location of Mutator insertions into the LKR/SDH gene was determined by sequencing of genomic DNA from individual mutants. An insertion mutation located in an exon in the LKR domain of the gene was chosen for further study. Southern blot analysis of corn genomic DNA indicated that corn contains only one LKR/SDH gene. Since an insertion mutation is expected to block function of the gene, it was anticipated that such a mutation would be recessive. One fourth of the progeny seed from a selfed corn ear with such a mutation segregating would be expected to be homozygous for the mutation. It was observed that approximately one fourth of such seed exhibited a higher level of free lysine than normal (5 to 15 fold higher) without the increase in the lysine catabolite saccharopine that is seen when free lysine is increased via expression of lysine insensitive DHDPS. It was concluded that knocking out LKR/SDH, by itself, was able to increase seed lysine content in corn seeds.

The LKR/SDH Mutator insertion line was crossed by a transgenic line that accumulates excess free lysine due to expression of lysine insensitive DHDPS and AK. In this cross two genetic loci that affect lysine accumulation, one of which is recessive (the LKR/SDH Mutator insertion) and one of which is semi-dominant (the lysine insensitive DHDPS and AK trangene locus), are segregating. Single seeds were analyzed for lysine and saccharopine content. The most striking observation from this experiment is that the highest lysine containing seeds have low levels of saccharopine (see figure). The low saccharopine level indicates that these seeds are homozygous for the LKR/SDH Mutator insertion, while the high lysine level indicates that they carry the lysine insensitive DHDPS and AK trangene locus. The level of lysine accumulation is considerably higher (2-3 fold) than the level provided by the

DHDPS and AK trangene locus alone. Thus, this experiment demonstrates that an increase in the accumulation of lysine, accompanied by a reduction in accumulation of lysine catabolites can be accomplished by combination of lysine overproduction brought about by expression of lysine insensitive DHDPS + AK and reduction of lysine catabolism by blocking expression of LKR/SDH, as we taught in the patent application. These results show that the concern stated in the Office Action on page 5 that "modifying metabolic pathways ... is highly unpredictable and often the desirable results are impossible to achieve" is unfounded in this particular case.

10. As indicated above, LKR/SDH expression has been blocked in corn via cosuppression. To accomplish this a chimeric gene designed for cosuppression of LKR was constructed by linking a 1268 bp LKR/SDH gene fragment, which included the LKR coding domain, to the corn endosperm 27 kD zein promoter and 10 kD zein 3' untranslated region. This chimeric gene was introduced into corn by particle-gun mediated transformation. Of 72 transformation events that were regenerated into plants and produced seed, 13 had seeds with a greater than four fold increase in free lysine. This is a typical frequency for cosuppression events. Since the transformed plants were out-crossed, the transgenic locus must be dominant or there would not have been any observable phenotype. This is expected from a cosuppression transgene, and is an advantage over knock-out mutations like the LKR/SDH Mutator insertion described above.

Some of the LKR cosuppression transformants have been carried forward for further testing. An event that has continued to show the increased free lysine phenotype for several generations and behaves genetically as a single locus transgene insertion has been selected for crossing to the transgenic line that accumulates excess free lysine due to expression of lysine insensitive DHDPS and AK. Results from that experiment are not yet available, but the expectation is that seeds carrying both transgene loci will have higher lysine levels than either parent, as was observed in the LKR Mutator insertion cross described above. In addition, co-transformation experiments in which the chimeric gene designed for cosuppression of LKR described above has been combined with a chimeric gene for expression of lysine insensitive DHDPS and introduced into corn by particle-gun mediated transformation are in progress. This is expected to yield transformants that produce seeds with the high lysine level observed in the LKR Mutator insertion cross by lysine insensitive DHDPS and AK, but with both chimeric genes at a single genetic locus, which is highly desirable for corn breeding.

In summary, all of the elements of the claimed invention were provided in the patent application. The teachings in this case are in the public domain, due to the issuance of U. S. Patent 5,773,691 of which the instant application claims priority as a divisional application.. One skilled in the art can take these elements, as discussed above, and practice the invention without undue experimentation.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Saverio Carl Falco

8/24/00

Date

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN THE APPLICATION OF:

SAVERIO C. FALCO
SHARON J. KEELER
JANET A. RICE

CASE NO.: BB-1037-D

APPLN. NO.: 08/823,771

GROUP ART UNIT: 1638

FILED: MARCH 24, 1997

EXAMINER: E. MCELWAIN

FOR: CHIMERIC GENES AND METHODS FOR INCREASING THE LYSINE AND THREONINE CONTENT OF THE SEEDS OF PLANTS

Date: FEBRUARY 16, 2001

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

Declaration of Dr. Carl Falco Pursuant to 37 CFR §1.132

I, Saverio Carl Falco, am a citizen of the United States of America, residing at 1902 Millers Road, Arden, Delaware 19810, United States of America, and I declare as follows:

1. I am one of the above-identified inventors named in this application. I am a graduate of Rutgers University of New Brunswick, New Jersey with a B.A. degree granted in 1971 with high honors and distinction in physics. I received a Ph.D. in 1977 from the University of Chicago in biochemistry and molecular biology. From 1977 to 1981 I was a National Institutes of Health postdoctoral fellow at the Massachusetts Institute of Technology. I have been employed by E. I. du Pont de Nemours and Company since 1981 directing and conducting research in plant genetic engineering.

2. I have reviewed the Office Action dated November 22, 2000. I am aware that this declaration is being submitted to address the concerns set forth on page 3 of the Office Action that the "Declaration of Falco teaches use of a bifunctional LKR/SDH gene to identify mutants produced by transposon mutagenesis. This plant does not contain a foreign LKR gene. In addition, the Declaration of Falco teaches of a combination DHDPS gene without an AK gene. Thus, the Declaration of Falco

does not teach a plant with a foreign LKR gene and a foreign DHDPS gene . . . it remains unpredictable what the results would be of introducing just the LKR gene and the DHDPS gene into a plant."

3. It was stated in paragraph 10 of my declaration previously submitted on August 24, 2000 that a co-transformation experiment in which a chimeric gene designed for co-suppression of LKR was combined with a chimeric gene for expression of lysine insensitive DHDPS was in progress. That experiment was expected to yield transformants that produced seeds with higher free lysine levels than transformants from a parallel experiment using the DHDPS gene alone. The results of those experiments have now been obtained and they do confirm the prediction that transformants comprising the chimeric gene designed for co-suppression of LKR and the chimeric gene for expression of lysine insensitive DHDPS produced seeds with higher free lysine levels than transformants from a parallel experiment using the DHDPS gene alone. These results are depicted in Figure 2 and Table 1.

4. The chimeric genes used for the experiments were:

i) corn globulin1 promoter/corn chloroplast transit sequence/ Corynebacterium dapA gene/corn globulin1 3'UTR; and

ii) corn 27kd zein promoter/fragment of corn LKR-SDH cDNA/corn 10kd zein 3' UTR

Seeds from many transformation events from each experiment were analyzed for free lysine content. It is clear from the data presented in Figure 2 that the best seeds obtained from the co-transformation experiment had considerably higher free lysine levels than the best seeds obtained from the transformation experiment where only the DHDPS gene was used. The average free lysine level from the 30 highest lysine seeds, or from the 70 highest lysine seeds, was about 2-fold higher for the co-transformation experiments compared the DHDPS only experiment.

5. It also was stated in paragraph 10 of my previous declaration submitted on August 24, 2000 that an LKR co-suppression transformant which showed an increased seed free lysine phenotype for several generations, and behaved genetically as a single locus transgene insertion, was crossed to a transgenic line that accumulates excess free lysine due to expression of lysine insensitive DHDPS and AK. Results from that experiment, which were not available at the time of the previous declaration, have confirmed the expectations expressed there, namely that seeds carrying both transgene loci will have higher free lysine levels than either parent. The data are presented in Figure 1.

6. In this experiment described in paragraph 5 above, transgenic lines homozygous for an insertion of DHDPS and AK genes, or homozygous for the co-suppressing LKR/SDH gene, were each crossed to a wild type corn line or to each

other. The F1 progeny seed from these crosses are hemizygous for the DHDPS and AK transgenic insertion, the co-suppressing LKR/SDH transgenic insertion, or both. Each cross was repeated at least 5 times, and seeds from the resulting corn ears were harvested and analyzed for free lysine levels. The results depicted in Figure 1 are averages derived from these repetitions. These results show the dramatic increase in free lysine resulting from the combination of increasing the synthesis of lysine via expression of the DHDPS gene and blocking the major pathway for lysine catabolism by co-suppressing the LKR/SDH gene.

7. Parenthetically, it is noted that a concern was raised in the Office Action dated November 22, 2000 that results from combining the DHDPS and AK transgenic insertions with a co-suppressing LKR/SDH transgenic insertion would not be predictive of combining a DHDPS only transgenic insertion with a co-suppressing LKR/SDH transgenic insertion. It is noted that there is evidence in the subject application that AK plays a secondary role to DHDPS for increasing the synthesis of lysine.

For example, it was demonstrated for (i) rapeseed transformants on page 31 at lines 18 – 24 of the specification that :

"Transformants expressing DHDPS protein showed a greater than 100-fold increase in free lysine level in their seeds. There was a good correlation between transformants expressing higher levels of DHDPS protein and those having higher levels of free lysine. One transformant that expressed AKIII-M4 in the absence of *Corynebacteria* DHDPS showed a 5-fold increase in the level of free threonine in the seeds. Concomitant expression of both enzymes resulted in accumulation of high levels of free lysine, but not threonine."

And for (ii) corn transformants (page 33 at lines 15 – 24:

"Free lysine levels in the seeds is increased from about 1.4% of free amino acids in control seeds to 15-27% in seeds of transformants expressing *Corynebacterium* DHDPS alone from the globulin 1 promoter. The increased free lysine was localized to the embryo in seeds expressing *Corynebacterium* DHDPS from the globulin 1 promoter.

The large increases in free lysine result in significant increases in the total seed lysine content. Total lysine levels can be increased at least 130% in seeds expressing *Corynebacterium* DHDPS from the globulin 1 promoter. . . . Greater increases in free lysine levels can be achieved by expressing *E. coli* AKIII-M4 protein from the globulin 1 promoter in concert with *Corynebacterium* DHDPS."

8. Thus, the gene encoding lysine insensitive AK can enhance the effect of the DHDPS gene on lysine synthesis by increasing overall flux through the biosynthetic pathway. However, AK does not increase lysine when expressed without DHDPS. It is the DHDPS gene that is necessary for increasing the synthesis of lysine. The presence of the AK gene along with the DHDPS gene in the cross described above is inconsequential with respect to proof of the concept that the **combination** of increasing lysine synthesis (which can be achieved using the DHDPS gene alone or in combination with the AK gene) and blocking lysine catabolism (which can be achieved by blocking expression of the LKR/SDH gene via co-suppression) works better than either alone.

9. The genetic cross experiment and the co-transformation experiment described above, taken together with the detailed description of the invention provided in the patent application and the previous declaration, clearly demonstrate that an increased lysine content is achieved when a lysine insensitive DHDPS gene (with or without a lysine insensitive AK gene) is combined with a co-suppressing LKR gene.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Saverio Carl Falco

Feb 16, 2001

Date

Figure 1: Compare DHDPS + AK, csLKR, DHDPS + AK + csLKR

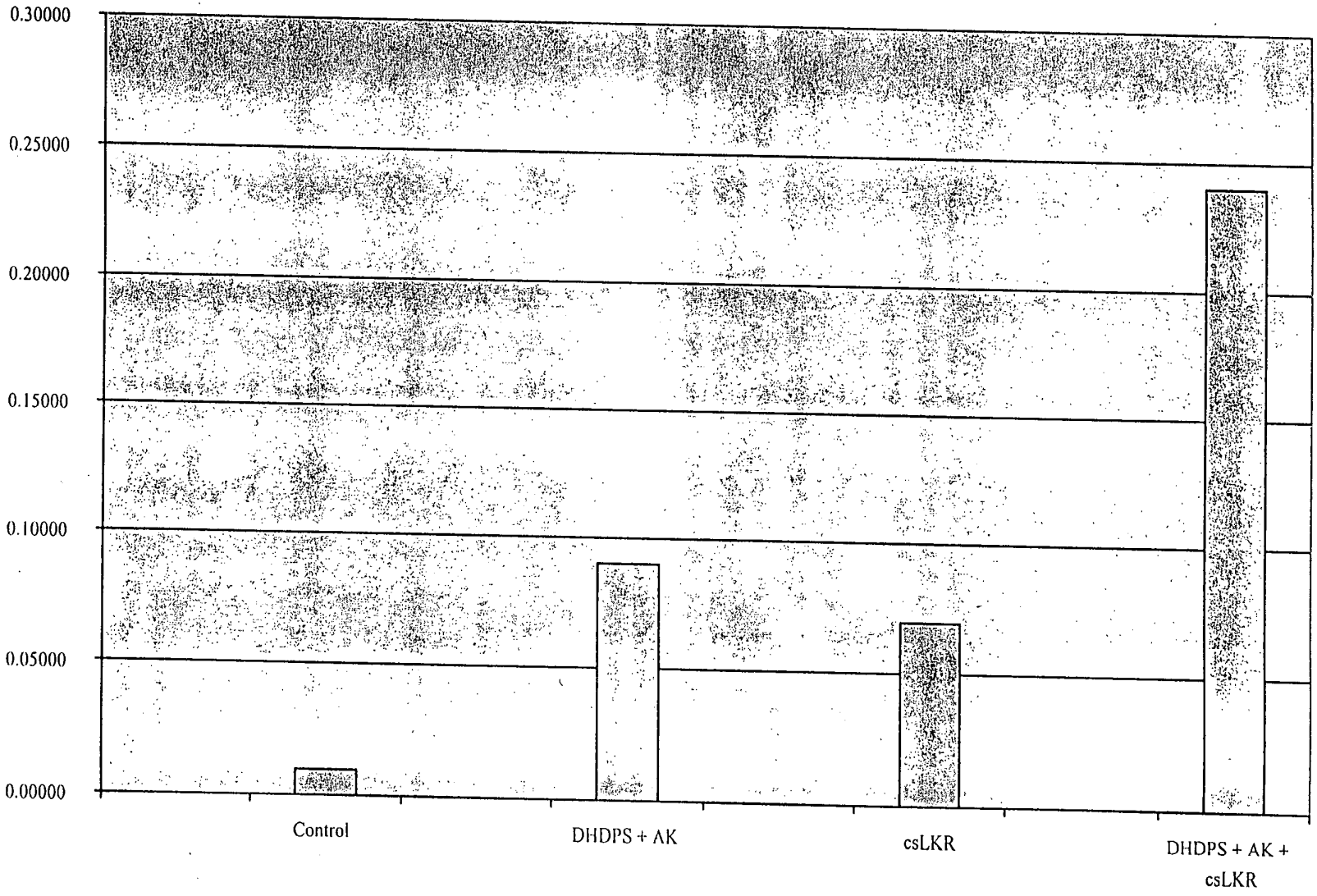

Figure 2: Comparison of seeds from transformation of DHDPS alone vs DHDPS + csLKR

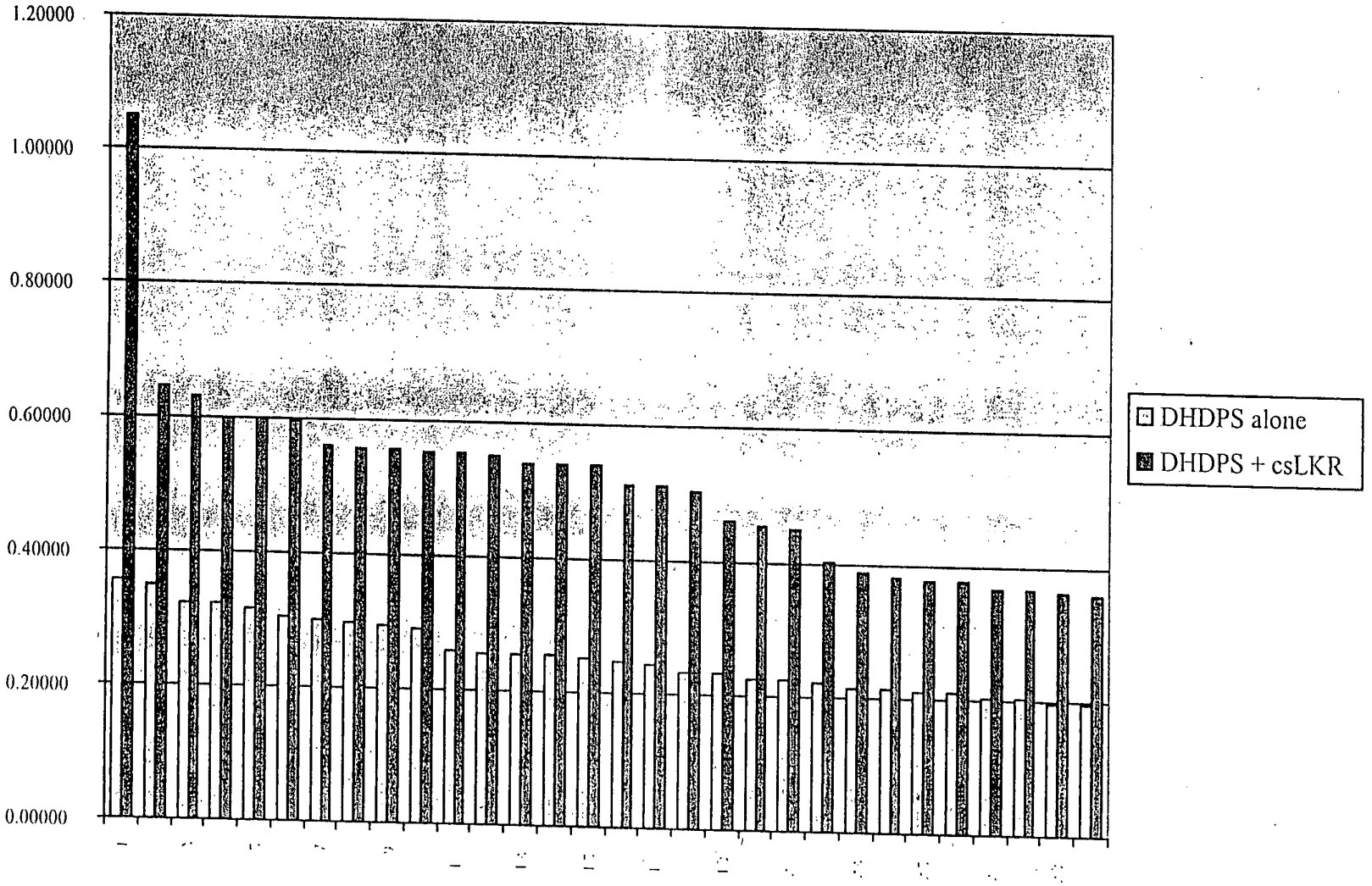

Table 1

	DHDPS alone wt % Free Lys	DHDPS + csLKR wt % Free Lys	wild type corn wt % Free Lys
Avg of best 30 seeds	**0.26**	**0.51**	**0.01**
Avg of best 70 seeds	**0.20**	**0.39**	**0.01**